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EXAMINER	
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ART UNIT	**PAPER NUMBER**
1637	

SHORTENED STATUTORY PERIOD OF RESPONSE	NOTIFICATION DATE	DELIVERY MODE
3 MONTHS	02/14/2007	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

Notice of this Office communication was sent electronically on the above-indicated "Notification Date" and has a shortened statutory period for reply of 3 MONTHS from 02/14/2007.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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Office Action Summary	Application No.	Applicant(s)	
	10/501,666	STORDEUR ET AL.	
	Examiner	Art Unit	
	Samuel Woolwine	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *13 November 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-39* is/are pending in the application.

4a) Of the above claim(s) *14-17,19,24 and 37-39* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-13,18,20-23 and 25-31* is/are rejected.

7)☒ Claim(s) *32-36* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *7/16/2004*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

Applicant's election of Group I, claims 1-13, 18, 20-23 and 25-36 in the reply filed on 11/13/2006 is acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)). Claims 14-17,19,24 and 37-39 are withdrawn from further consideration. This requirement for restriction is made FINAL.

Claim Objections

Claims 32-36 are objected to under 37 CFR 1.75(c) as being in improper form because a multiple dependent claim cannot depend from any other multiple dependent claim; note claim 32 depends from any of claims 25-31, and claim 25 is a multiple dependent claim. See MPEP § 608.01(n). Accordingly, the claims 32-36 have not been further treated on the merits.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 5, 8, 10 and 13 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

Claims 5 and 13 contain the trademark/trade name PAXgene™. Where a trademark or trade name is used in a claim as a limitation to identify or describe a

particular material or product, the claim does not comply with the requirements of 35 U.S.C. 112, second paragraph. See *Ex parte Simpson*, 218 USPQ 1020 (Bd. App. 1982). The claim scope is uncertain since the trademark or trade name cannot be used properly to identify any particular material or product. A trademark or trade name is used to identify a source of goods, and not the goods themselves. Thus, a trademark or trade name does not identify or describe the goods associated with the trademark or trade name. In the present case, the trademark/trade name is used to identify/describe a material, i.e. a compound inhibiting cellular RNA degradation and/or gene induction (claim 5), and a product, i.e. a tube for collecting a biological sample (claim 13), and, accordingly, the identification/description is indefinite.

Claim 8 contains the trademark/trade name MagNa Pure LC mRNA Isolation Kit I. Where a trademark or trade name is used in a claim as a limitation to identify or describe a particular material or product, the claim does not comply with the requirements of 35 U.S.C. 112, second paragraph. See *Ex parte Simpson*, 218 USPQ 1020 (Bd. App. 1982). The claim scope is uncertain since the trademark or trade name cannot be used properly to identify any particular material or product. A trademark or trade name is used to identify a source of goods, and not the goods themselves. Thus, a trademark or trade name does not identify or describe the goods associated with the trademark or trade name. In the present case, the trademark/trade name is used to identify/describe a material, i.e. a lysis buffer, and, accordingly, the identification/description is indefinite.

Claim 10 contains the trademark/trade name MagNA Pure LC Instrument. Where a trademark or trade name is used in a claim as a limitation to identify or describe a particular material or product, the claim does not comply with the requirements of 35 U.S.C. 112, second paragraph. See *Ex parte Simpson*, 218 USPQ 1020 (Bd. App. 1982). The claim scope is uncertain since the trademark or trade name cannot be used properly to identify any particular material or product. A trademark or trade name is used to identify a source of goods, and not the goods themselves. Thus, a trademark or trade name does not identify or describe the goods associated with the trademark or trade name. In the present case, the trademark/trade name is used to identify/describe a product, i.e. an automated device, and, accordingly, the identification/description is indefinite.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1-4, 6, 7, 12 and 20-22 are rejected under 35 U.S.C. 102(b) as being anticipated by Hamel et al (JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1995, Vol. 33, No. 2, p. 287–291).

With regard to claim 1, Hamel teaches a method for the quantification of in vivo RNA from a biological sample comprising the steps of:

(a) collecting said biological sample in a tube comprising a compound inhibiting RNA degradation and/or gene induction (See page 288, column 1, "RNA extraction": "Two drops of blood containing sodium citrate or heparin or four drops of serum, other fluids, or swab suspensions were vortex mixed with 0.5 ml of Cat-14 containing 1 drop of yeast RNA." Cat-14 is tetradecyltrimethyl-ammonium oxalate (see page 287, last paragraph preceding "Materials and Methods"), which according to claim 4, must be *a compound which inhibits RNA degradation and/or gene induction*. One of ordinary skill in the art would have reasonably inferred this was done in some sort of "tube", since the samples were vortexed, centrifuged and inverted to drain off the supernatant (see page 288, column 1, "RNA extraction"),

(b) forming a precipitate comprising nucleic acids (See page 288, column 1, "RNA extraction": "For all types of specimens, after the addition of Cat-14 and yeast RNA, samples were immediately vortex mixed for about 30 s and were left at room temperature for 10 to 30 min." This evidently forms a precipitate, as evidenced by the text cited for the next limitation.),

(c) separating said precipitate of step (b) from the supernatant (See page 288, column 1, "RNA extraction": "Samples were then microcentrifuged for 5 min, drained by inverting them, and briefly microcentrifuged (5 s). Any remaining supernatant was removed with filter barrier micropipet tips..."),

(d) dissolving said precipitate of step (c) using a buffer, forming a suspension (See page 288, column 1, "RNA extraction": "...and the pellets (containing precipitated Cat-14 salt of RNA and DNA) were dissolved in 0.2 ml of GITC buffer (4 M guanidine

thiocyanate, 0.2 M sodium acetate [pH 4.0], 0.1 M 2-mercaptoethanol), vortex mixed (intermittently for up to 5 min), and then kept on ice."),

(e) isolating nucleic acids from said suspension of step (d) using an automated device (See page 288, column 1, "RNA extraction": "For each extraction, samples were vortex mixed for 30 s and microcentrifuged for 5 min at 48C." Vortex mixers and microcentrifuges are *automated devices*.),

(f) dispersing/distributing a reagent mix for RT-PCR using an automated device (See page 288, column 2, "RT-PCR": "In order to minimize pipetting errors..." and "...only filter barrier micropipette tips were used...". This implicitly teaches micropipettes, which are *automated devices*.),

(g) dispersing/distributing the nucleic acids isolated in step (e) within the dispersed reagent mix of step (f) using an automated device (See page 288, column 2, "RT-PCR": "In order to minimize pipetting errors..." and "...only filter barrier micropipette tips were used...". This implicitly teaches micropipettes, which are *automated devices*.), and,

(h) determining the in vivo levels of transcripts using the nucleic acid/RT-PCR reagent mix of step (g) in an automated setup (See page 288, column 2, "RT-PCR": "RT-PCR was performed on a programmable thermocycler." A programmable thermocycler is an *automated setup*, which *uses the nucleic acid/RT-PCR reagent mix*.).

With regard to claim 2, see page 288, column 1, "RNA extraction": "For all types of specimens, after the addition of Cat-14 and yeast RNA, samples were immediately vortex mixed for about 30 s and were left at room temperature for 10 to 30 min." This

evidently forms a precipitate, as evidenced by the text cited for limitation (c) of claim 1. Therefore, collection of the sample in a tube with the compound and the formation of a precipitate occurred *simultaneously*.

With regard to claim 3, Cat-14 is tetradecyltrimethyl-ammonium oxalate (see page 287, last paragraph preceding "Materials and Methods"), which according to claim 4, must be a *quaternary amine surfactant*.

With regard to claim 4, Cat-14 is *tetradecyltrimethyl-ammonium oxalate* (see page 287, last paragraph preceding "Materials and Methods").

With regard to claim 6, it has already been stated in the discussion of claim 1 above that one of ordinary skill in the art would have reasonably inferred that the samples were manipulated in tubes, since the samples were vortexed, centrifuged and inverted to drain off the supernatant (see page 288, column 1, "RNA extraction"). The term "tube" and "blood collection tube" are not explicitly defined in the specification, and thus the term "blood collection tube" does not structurally distinguish over the "tube" implicitly taught by Hamel. Regarding the tube being "*open*" or "*closed*", the tubes implicitly taught by Hamel had to have been either open or closed, or intermittently opened and closed; there is no other logical possibility.

With regard to claim 7, Hamel teaches *guanidine-thiocyanate-containing buffer* (see page 288, column 1, "RNA extraction": "...and the pellets (containing precipitated Cat-14 salt of RNA and DNA) were dissolved in 0.2 ml of GITC buffer (4 M guanidine thiocyanate, 0.2 M sodium acetate [pH 4.0], 0.1 M 2-mercaptoethanol), vortex mixed (intermittently for up to 5 min), and then kept on ice.").

With regard to claim 12, Hamel teaches using a biological sample of 100 μl (see page 288, column 2, "RT-PCR detection limit": "Total RNA was extracted, with and without added carrier yeast RNA, from duplicate 100 μl aliquots of several viral dilutions...").

With regard to claim 20, Hamel teaches *a method for the monitoring/detection of changes of in vivo nucleic acids levels in a biological agent present in a biological sample according to claim 1* (see page 290, column 1, first paragraph: "After only a few successive passages (usually no more than five), our negative control cell cultures consistently tested positive for BVDV (assayed by FAT and RT-PCR) and displayed increasing levels of intensity after successive passages (data not shown).").

With regard to claim 21, Hamel teaches detection of bovine viral diarrhea virus (see title).

With regard to claim 22, Hamel teaches his method is "an alternative to the conventional cell culture assays used in a diagnostic laboratory and is an improvement over existing RT-PCR assays for BVDV" (see abstract). One of ordinary skill in the art would have reasonably inferred Hamel's method as a method of diagnosing BVDV.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 9 and 11 are rejected under 35 U.S.C. 103(a) as being unpatentable over Winer et al (Analytical Biochemistry 270, 41–49 (1999)) in view of Hamel et al (JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1995, Vol. 33, No. 2, p. 287–291).

With regard to claim 9, Winer teaches isolation of nucleic acids using RNA-capturing beads (see page 42, column 1, "mRNA Preparation").

With regard to claim 11, Winer teaches determination of in vivo levels of RNA using real time PCR (see page 41, column 2, first sentence of second paragraph for

example: "In this article, we validate the use of this real-time RT–PCR method to analyze gene expression...").

Winer does not teach the method according to claim 1 or 2, as required by claims 9 and 11.

Hamel teaches the method of claims 1 and 2 as discussed above in the rejections under 35 U.S.C. 102, i.e. a method for the quantification of in vivo RNA from a biological sample comprising the steps of:

(a) collecting said biological sample in a tube comprising a compound inhibiting RNA degradation and/or gene induction (See page 288, column 1, "RNA extraction": "Two drops of blood containing sodium citrate or heparin or four drops of serum, other fluids, or swab suspensions were vortex mixed with 0.5 ml of Cat-14 containing 1 drop of yeast RNA." Cat-14 is tetradecyltrimethyl-ammonium oxalate (see page 287, last paragraph preceding "Materials and Methods"), which according to claim 4, must be *a compound which inhibits RNA degradation and/or gene induction.* One of ordinary skill in the art would have reasonably inferred this was done in some sort of "tube", since the samples were vortexed, centrifuged and inverted to drain off the supernatant (see page 288, column 1, "RNA extraction"),

(b) forming a precipitate comprising nucleic acids (See page 288, column 1, "RNA extraction": "For all types of specimens, after the addition of Cat-14 and yeast RNA, samples were immediately vortex mixed for about 30 s and were left at room temperature for 10 to 30 min." This evidently forms a precipitate, as evidenced by the text cited for the next limitation.),

(c) separating said precipitate of step (b) from the supernatant (See page 288, column 1, "RNA extraction": "Samples were then microcentrifuged for 5 min, drained by inverting them, and briefly microcentrifuged (5 s). Any remaining supernatant was removed with filter barrier micropipet tips..."),

(d) dissolving said precipitate of step (c) using a buffer, forming a suspension (See page 288, column 1, "RNA extraction": "...and the pellets (containing precipitated Cat-14 salt of RNA and DNA) were dissolved in 0.2 ml of GITC buffer (4 M guanidine thiocyanate, 0.2 M sodium acetate [pH 4.0], 0.1 M 2-mercaptoethanol), vortex mixed (intermittently for up to 5 min), and then kept on ice."),

(e) isolating nucleic acids from said suspension of step (d) using an automated device (See page 288, column 1, "RNA extraction": "For each extraction, samples were vortex mixed for 30 s and microcentrifuged for 5 min at 48C." Vortex mixers and microcentrifuges are *automated devices.*),

(f) dispersing/distributing a reagent mix for RT-PCR using an automated device (See page 288, column 2, "RT-PCR": "In order to minimize pipetting errors..." and "...only filter barrier micropipette tips were used...". This implicitly teaches micropipettes, which are *automated devices.*),

(g) dispersing/distributing the nucleic acids isolated in step (e) within the dispersed reagent mix of step (f) using an automated device (See page 288, column 2, "RT-PCR": "In order to minimize pipetting errors..." and "...only filter barrier micropipette tips were used...". This implicitly teaches micropipettes, which are *automated devices.*), and,

(h) determining the in vivo levels of transcripts using the nucleic acid/RT-PCR reagent mix of step (g) in an automated setup (See page 288, column 2, "RT-PCR": "RT-PCR was performed on a programmable thermocycler." A programmable thermocycler is an *automated setup*, which *uses the nucleic acid/RT-PCR reagent mix*.).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention of the instant application was made to use the method of claims 1 or 2 as taught by Hamel to isolate the mRNA in the method of Winer, incorporating the use of oligo(dt) beads for capturing the mRNA, which Winer teaches "minimized DNA contamination" (see Winer abstract). One would have been motivated to use Hamel's method to isolate the mRNA because Hamel expressly suggests using his protocol for gene expression studies, which is precisely what Winer is concerned with, i.e. studying gene expression. Hamel states on page 291, last paragraph of the discussion: "In addition, our method should prove to be useful for other investigations involving RNA, such as for the detection of other RNA viruses and for gene expression studies."

Claims 18 is rejected under 35 U.S.C. 103(a) as being unpatentable over Hamel et al (JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1995, Vol. 33, No. 2, p. 287–291) in view of Walker (J BIOCHEM MOLECULAR TOXICOLOGY, Volume 15, Number 3, 2001).

With regard to claim 18, Hamel teaches all the limitations of claim 1, as discussed in the rejection of claim 1 under 35 U.S.C. 102 above, but does not teach skipping the RT reaction or quantifying DNA.

Walker teaches quantifying DNA by PCR (see entire document, for example, section entitled "RTAQ-PCR METHODOLOGY", beginning on page 121).

It would have been *prima facie* obvious to use Hamel's method to quantify DNA as well as RNA. It would have been obvious because Hamel teaches that Catrimox-14 precipitates DNA as well as RNA (see page 288, column 1, "RNA extraction": "Any remaining supernatant was removed with filter barrier micropipet tips, and the pellets (containing precipitated Cat-14 salt of RNA and DNA) were dissolved in 0.2 ml of GITC buffer)"), and because Walker points out clear motivations for quantifying DNA (for example, page 124, column 2, "DNA Analysis: Molecular Genetics" and "Exposure Monitoring").

Claims 23 and 25-31 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kammula et al (J Natl Cancer Inst 2000;92:1336–44) in view of Hamel et al (JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1995, Vol. 33, No. 2, p. 287–291).

With regard to claim 23, Kammula teaches *a method for the monitoring/detection of changes of in vivo nucleic acids of a biological agent* (PBMCs) *in a biological sample* (see page 1337, column 1, 3rd paragraph: "In this study, we have used a sensitive, quantitative, real-time polymerase chain reaction (PCR) assay to directly assess the immune status of PBMCs from patients prior to any in vitro stimulation with antigen"; i.e. *in vivo* changes of nucleic acids), *in order to screen for a compound for the production of a medicament for curing a disease* (see page 1337, column 1, 2nd paragraph: "...treating patients with metastatic melanoma with a synthetically modified melanoma

peptide..." and see page 1339, last paragraph in column 1 continuing in column 2, especially text cited for claim 27 below). Kammula was screening a potential vaccine for "curing" melanoma.

With regard to claim 25, melanoma is cancer, which according to claim 32 of the instant application, is an "immuno-related disease".

With regard to claim 26, Kammula teaches a vaccine (which is also a peptide) (see page 1339, last paragraph in column 1 continuing in column 2, especially text as cited for claim 27 below).

With regard to claim 27, Kammula teaches *a method for the detection/monitoring of epitope specific CTLs* **and** *immuno-related transcripts* (see page 1339, last paragraph in column 1 continuing in column 2):

> "To develop an assay that directly detects specific antigen recognition and reactivity by CTLs in peripheral blood, we obtained PBMCs from a patient before and after two rounds of g209-2M vaccination... As shown in Fig. 1, c, preimmunization PBMCs demonstrated no detectable changes in IFN γ mRNA (after normalization to CD8 mRNA) after g209 peptide exposure. However, postimmunization PBMCs showed a marked increase in IFN γ mRNA, with peak expression between 2 and 3 hours after peptide exposure. In addition, we noted that the g209 peptide induced gene expression for the CD69 representing a marker of CTL activation, the IL-2α receptor (CD25), and the cytokines tumor necrosis factor-α, GM-CSF, and IL-2. Control peptides resulted in no change in cytokine mRNA expression in either the preimmunized or postimmunized samples (data not shown)." (citations omitted)

The fact that control peptides resulted in no change in cytokine mRNA expression demonstrates that, in addition to detecting immuno-related transcripts, Kammula was monitoring epitope specific CTLs.

With regard to claim 28, Kammula teaches *a method to identify an agent capable of modifying the immunological status of a subject via the analysis of epitope specific CTLs comprising the steps of:*

(a) applying an immunomodulatory agent(s) into a subject (see page 1339, last paragraph in column 1 continuing in column 2: "To develop an assay that directly detects specific antigen recognition and reactivity by CTLs in peripheral blood, we obtained PBMCs from a patient before and after two rounds of g209-2M vaccination."),

(b) sampling whole blood from said subject (see page 1339, last paragraph in column 1 continuing in column 2: "To develop an assay that directly detects specific antigen recognition and reactivity by CTLs in peripheral blood, we obtained PBMCs from a patient before and after two rounds of g209-2M vaccination." Note PBMC stands for peripheral blood mononuclear cells. Although Kammula separates out the PBMCs from whole blood, he nevertheless necessarily sampled whole blood from his subjects.),

(c) optionally, pulsing blood cells present in the whole blood sample of step (b) with an identical/similar and/or different immunomodulatory agent as applied in step (a) (see page 1338, column 1, "In Vitro Sensitization Assay of Peptide and Melanoma-Specific CTL Reactivity": "The harvested cells were then stimulated with melanoma cells or the antigen-processing-defective, HLA-A*0201-expressing T2 cells pulsed with 1 μM g209 peptide or a control peptide g154 for 18–24 hours at 37 °C." (citations omitted)),

(k) detecting/monitoring/analyzing the in vivo levels of epitope specific CTLs-related transcripts in the dispersed solution of step (j) in an automated setup (see page 1338, column 1, "Quantitative Real-Time PCR": "Gene expression was measured with the use of the ABI Prism 7700 Sequence Detection System"; this is an automated setup),

(l) identifying agents able to modify the immunological status of said subject, whereby, in case the agent of step (a) is already present in the subject, step (a) is omitted (see page 1339, column 2:

> "However, postimmunization PBMCs showed a marked increase in IFN γ mRNA, with peak expression between 2 and 3 hours after peptide exposure. In addition, we noted that the g209 peptide induced gene expression for the CD69 representing a marker of CTL activation, the IL-2α receptor (CD25), and the cytokines tumor necrosis factor-α, GM-CSF, and IL-2. Control peptides resulted in no change in cytokine mRNA expression in either the preimmunized or postimmunized samples (data not shown)." (citations omitted)

thus, Kammula identified the g209 vaccine as an agent able to modify the immunological status of a subject).

With regard to claim 29, the only difference between this claim and claim 28 is the more general detection of immuno-related transcripts, rather than the more specific detection of epitope specific CTLs-related transcripts (compare step (k) of each claim). In any event, Kammula teaches *detecting/monitoring/analyzing the in vivo levels of immuno-related transcripts* (see discussion of step (k) for claim 28).

With regard to claim 30, Kammula teaches *monitoring of a clinical status affecting the immune system in a subject comprising the steps of (a) sampling whole blood from said subject*, as discussed for claim 28 above.

With regard to claim 31, Kammula teaches *monitoring of a clinical status affecting the immune system in a subject comprising the steps of (a) sampling whole blood from said subject* and *(b) pulsing blood cells present in the whole blood sample with an identical/similar and/or different immunomodulatory agent as present in the subject* as discussed for claim 28 above.

Kammula does not teach certain steps in the method of claim 1 (as required by claims 23, 25 and 26. Kammula does not teach certain of the method steps of claims 28-31. These steps are (as stated in claim 28):

(d) collecting pulsed blood cells of step (c) or non-pulsed blood cells of step (b) in a tube comprising a compound inhibiting RNA degradation and/or gene induction, or adding said compound to the pulsed/non-pulsed cells,

(e) forming a precipitate comprising nucleic acids,

(f) separating said precipitate of step (e) from the supernatant,

(g) dissolving said precipitate of step (f) using a buffer, forming a suspension,

(h) isolating nucleic acids from said suspension of step (g) using an automated device,

(i) dispersing/distributing a reagent mix for RT-PCR using an automated device,

(j) dispersing/distributing the nucleic acids isolated in step (h) within the dispersed reagent mix of step (i) using an automated device.

Hamel teaches the method of claim 1 as discussed in the rejection under 35 U.S.C. 102 above. In particular Hamel teaches a method for the quantification of in vivo RNA from a biological sample comprising the steps of:

collecting said biological sample in a tube comprising a compound inhibiting RNA degradation and/or gene induction (See page 288, column 1, "RNA extraction": "Two drops of blood containing sodium citrate or heparin or four drops of serum, other fluids, or swab suspensions were vortex mixed with 0.5 ml of Cat-14 containing 1 drop of yeast RNA." Cat-14 is tetradecyltrimethyl-ammonium oxalate (see page 287, last paragraph

preceding "Materials and Methods"), which according to claim 4, must be *a compound which inhibits RNA degradation and/or gene induction*. One of ordinary skill in the art would have reasonably inferred this was done in some sort of "tube", since the samples were vortexed, centrifuged and inverted to drain off the supernatant (see page 288, column 1, "RNA extraction"),

forming a precipitate comprising nucleic acids (See page 288, column 1, "RNA extraction": "For all types of specimens, after the addition of Cat-14 and yeast RNA, samples were immediately vortex mixed for about 30 s and were left at room temperature for 10 to 30 min." This evidently forms a precipitate, as evidenced by the text cited for the next limitation.),

separating said precipitate from the supernatant (See page 288, column 1, "RNA extraction": "Samples were then microcentrifuged for 5 min, drained by inverting them, and briefly microcentrifuged (5 s). Any remaining supernatant was removed with filter barrier micropipet tips..."),

dissolving said precipitate of step using a buffer, forming a suspension (See page 288, column 1, "RNA extraction": "...and the pellets (containing precipitated Cat-14 salt of RNA and DNA) were dissolved in 0.2 ml of GITC buffer (4 M guanidine thiocyanate, 0.2 M sodium acetate [pH 4.0], 0.1 M 2-mercaptoethanol), vortex mixed (intermittently for up to 5 min), and then kept on ice."),

isolating nucleic acids from said suspension using an automated device (See page 288, column 1, "RNA extraction": "For each extraction, samples were vortex mixed

for 30 s and microcentrifuged for 5 min at 48C." Vortex mixers and microcentrifuges are *automated devices*.),

dispersing/distributing a reagent mix for RT-PCR using an automated device (See page 288, column 2, "RT-PCR": "In order to minimize pipetting errors..." and "...only filter barrier micropipette tips were used...". This implicitly teaches micropipettes, which are *automated devices*.),

dispersing/distributing the nucleic acids within the dispersed reagent mix using an automated device (See page 288, column 2, "RT-PCR": "In order to minimize pipetting errors..." and "...only filter barrier micropipette tips were used...". This implicitly teaches micropipettes, which are *automated devices*.).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention of the instant application was made to use the method of Hamel to isolate the mRNA in the method of Kammula. One would have been motivated to use Hamel's method to isolate the mRNA because Hamel expressly suggests using his protocol for gene expression studies, which is precisely what Kammula is concerned with, i.e. studying gene expression. Hamel states on page 291, last paragraph of the discussion: "In addition, our method should prove to be useful for other investigations involving RNA, such as for the detection of other RNA viruses and for gene expression studies."

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Samuel Woolwine whose telephone number is (571) 272-1144. The examiner can normally be reached on Mon-Fri 9:00am-5:00pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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scw

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